REMARKS and REQUEST FOR RECONSIDERATION

Claims 1, 6-9 and 11-26 are currently pending in the application. Claims 1, 6-9 and 11-26 are rejected. Claim 1 is newly amended. The amendment finds support in the specification and is discussed in the relevant sections below. No new matter is added.

Claim Rejections-35 USC § 102

The Office Action states that Claims 1, 7, 11-12 and 14-18 are rejected under 35 U.S.C. 102(e) as being anticipated by Howley et al. (US 6,432,926).

The Office Action states that

> "Howley et al. teaches a method of preparing a nucleic acid sample for an analytical procedure, said sample comprising template nucleic acid and synthetic nucleic acid, wherein said template and synthetic nucleic acid comprise DNA, said method comprising treating said sample with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid, and subjecting said treated sample to an analytical procedure, wherein said analytical procedure is selected from the group consisting of gel electrophoresis and Southern blotting".

Proposed newly amended Claim 1 is no longer recites the analytical procedures of gel electrophoresis and Southern blotting. And Howley et al. does not teach a method comprising the analytical procedures of the claimed invention, i.e. wherein said analytical procedure is selected from the group consisting of anion-exchange chromatography, size-exclusion chromatography, pulse-field electrophoresis, polyacrylamide gel electrophoresis, sieving gel electrophoresis, and Northern analysis, as required by proposed newly amended claim.

Since proposed newly amended Claim 1 and its dependent claims 7, 11-12 and 14-18 no longer recites a method of preparing a nucleic acid sample comprising the analytical procedures of gel electrophoresis and Southern blotting, Howley et al. does not anticipate claim 1, as newly amended, and its dependent claims 7, 11-12 and 14-18. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

Claims 1, 7 and 11-12 are rejected under 35 U.S.C. 102(e) as being anticipated by Dayn et al. (6,245,565).

Applicant traverses the rejection on the grounds that the cited art does not teach each and every claim limitation of newly amended claim 1, from which the other claims depend.

The Office Action states that:

> "Dayn et al. teaches a method of preparing a nucleic acid sample for an analytical procedure, said sample comprising template nucleic acid and synthetic nucleic acid, wherein said template and synthetic nucleic acid comprise DNA, said method comprising treating said sample with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid, and subjecting said treated sample to an analytical procedure, wherein said analytical procedure is selected from the group consisting of gel electrophoresis and Southern blotting".

Proposed newly amended Claim 1 is no longer recites the analytical procedures of gel electrophoresis and Southern blotting. And Dayn et al. does not teach a method comprising the analytical procedures of the claimed invention, i.e. wherein said analytical procedure is selected from the group consisting of anion-exchange chromatography, size-exclusion chromatography, pulse-field electrophoresis, polyacrylamide gel electrophoresis, sieving gel electrophoresis, and Northern analysis, as required by proposed newly amended claim.

Since proposed newly amended Claim 1 and its dependent claims 7, and 11-12, no longer recite the analytical procedures of gel electrophoresis and Southern blotting, Dayne et al. does not anticipate claim 1, as newly amended, and its dependent claims 7, and 11-12. Accordingly, reconsideration and withdrawal of the rejection is respectfully requested.

The Office Action states that Claims 6, 9, 19 and 23-24 are rejected under 35 U.S.C. 102(b) as being anticipated by Gong et al.

The Office action also states that:

> "Gong et al. teaches a transcription reaction wherein a nucleic acid sample is generated comprising template nucleic acid and synthetic RNA, the improvement whereby after the transcription reaction and immediately prior to the analysis of the RNA sample, said sample is treated with a substance that cleaves the template nucleic acid and does not substantially cleave the RNA, wherein said substance is a restriction enzyme".

The Office Action refers to pages 539 and 541 of Gong et al. as teaching;

> "(...the treatment of the restriction enzyme Dnase I cleaves the DNA template and does not substantially cleave the synthetic RNA)".

Applicant traverses the rejection on the grounds that Gong et al. does not teach each and every claim limitation. Specifically, Gong et al does not teach the limitation "wherein said substance is a restriction enzyme", in referring to the substance that cleaves the template nucleic acid and does not substantially cleave the RNA. Gong teaches a method wherein DNAse I is the substance that cleaves the template nucleic acid and does not substantially cleave the RNA. However, DNAse I is not a restriction enzyme.

DNAse I is deoxyribonuclease I, and is a nuclease, specifically a non-sequence-specific endonuclease. Deoxyribonuclease I degrades single-stranded or double-stranded DNA to produce 3′-hydroxyl oligonucleotides, see Exhibit 'A', which is an attached page from Promega Catalog. As a non-sequence-specific endonuclease, DNAse I is not a restriction enzyme because restriction enzymes are sequence specific as disclosed in USPN 6,521,409 cited by the Examiner in the instant Office Action. Specifically, '409 defines restriction enzymes in column 14, lines 41-45, as: " naturally occurring enzymes with the ability to recognize a particular arrangement of nucleotide bases and, with absolute specificity, to cleave double stranded DNA at or near the site of recognition".

Because Gong et al. does not teach the instantly claimed method comprising a restriction enzyme, as recited in claim 6 and its dependent claims 9, 19 and 23-24, Gong et al. does not anticipate the claimed invention. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection.

The Office Action states that Claims 6, 9, 19 and 23-24 are rejected under 35 U.S.C. 102(b) as being anticipated by Chung et al.

The Office Action also states that:

> "Chung et al. teaches a transcription reaction wherein a nucleic acid sample is generated comprising template nucleic acid and synthetic RNA, the improvement whereby after the transcription reaction and

> immediately prior to the analysis of the RNA sample, said sample is treated with a substance that cleaves the template nucleic acid and does not substantially cleave the RNA, wherein said substance is a restriction enzyme".

The Office Action refers to column 8 of Chung et al. as teaching "(..the treatment of the restriction enzyme Dnase I cleaves the DNA template and does not substantially cleave the synthetic RNA)".

Applicant traverses the rejection on the grounds that Chung et al. does not teach every limitation. Specifically, Chung et al. does not teach the limitation "wherein said substance is a restriction enzyme", wherein the substance cleaves the template nucleic acid and does not substantially cleave the RNA. Chung teaches that the substance that cleaves the template nucleic acid and does not substantially cleave the RNA, is DNAse I. However, as discussed above, DNAse I is not a restriction enzyme.

Because Chung et al. does not teach the instantly claimed method comprising a restriction enzyme, as recited in claim 6 and its dependent claims 9, 19 and 23-24, Chung et al. does not anticipate the claimed invention. Accordingly, Applicant respectfully requests reconsideration and withdrawal of the rejection.

Rejections under 103(a)

The Office Action states that Claims 1, 7-9, 11-12 and 14-18 are rejected under 35 U.S.C. 103(a) as being unpatentable over Bauer et al. (5,789,166) in view of Yuan (6,376,210), in further view of Prober et al. (Science (1987) 238:336-341).

Applicant traverses the inclusion of claim 9 in the instant rejection , since claim 9 depends from independent claim 6, not from any Claim 1, 7 or 8. Applicant notes that the method of claim 9 is not addressed in the instant rejection in a way that is meaningful to its claimed method.

Applicant traverses the rejection on the grounds that the cited references, either singularly or when combined, do not teach the claimed invention. The claims require that the

sample subjected to an analytical procedure is "said treated sample", (see lines 5-6 of Claim 1), that "said sample comprises template nucleic acid and synthetic nucleic acid", (see lines 2-3 of claim 1), and that said sample is "treated with a substance that cleaves template nucleic acid without substantially cleaving said synthetic nucleic acid", (see lines 4-5 of claim 1).

The Office Action states that

> "Bauer et al. teach a method for preparing a nucleic sample for an analytical procedure (mutagenesis), said method comprising treating said sample with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid",

and

> "Bauer et al teaches that following the digestion the mutagenized DNA (the prepared nucleic acid) is transformed",

and

> "Bauer et al does not teach preparing the nucleic acid for an analytical procedure such as gel electrophoresis for use in sequencing the prepared DNA",

The Office Action further states that:

> "Yuan also teaches a method of mutatgenesis , wherein after transforming the prepared nucleic acid, the transformed colonies are confirmed by sequencing.....Accordingly Yuan teaches it is advantageous to confirm the transformed colonies by sequencing."

and

> "..in view of the teachings of Yuan and Prober, it would have been obvious to one of ordinary skill at the time the invention was made to have modified the method of Bauer so as to have carried out a sequencing reaction comprising the use of fluorescent chain terminating dideooxynucleotides coupled with polyacrylamide gel electropohoresis, ***after transforming the prepared nucleic acid***", (emphasis added).

Applicant contends that the teachings of Yuan and Prober do not make up the deficiencies of Bauer with respect to the claimed invention, and therefore the combination of the three cited references do not arrive at the claimed invention. The instant claims require that the nucleic acid sample that is subjected to an analytical procedure and treated with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid, ***comprise template nucleic acid and synthetic nucleic acid***. In contrast, Yuan teach that ***a***

sample of DNA from transformed colonies is treated with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid and subjected to an analytical procedure. A sample of DNA from transformed colonies does not contain template nucleic acid as well as synthetic nucleic acid.

Nor do the combined teachings of Yuan and Prober produce the claimed invention because the sample taught to be subjected to the analytical procedure is not the "treated sample" comprising template nucleic acid and synthetic nucleic acid as required by the instant claims. The instant claims recite a method of preparing a nucleic acid sample for an analytical procedure, wherein said sample comprises a template and a synthetic nucleic acid. The method comprises two steps. The first step is "...treating said sample with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid....". The second step is "subjecting ***said treated sample*** to an analytical procedure...".

Therefore, the sample of nucleic acid subjected to the analytical procedures comprising polyacrylamide gel electrophoresis of a sequencing reaction as taught by the combined references of Yuan and Prober and Bauer, is not the recited "treated sample" required by the instant claims. Instead, in the obviousness rejection, the sample of nucleic acid subjected to the analytical procedure comprising polyacrylamide gel electrophoresis of a sequencing reaction, is nucleic acid that has been isolated from transformed cells. Nucleic acid from transformed cells is distinct from the claimed nucleic acid sample comprising a template and a synthetic nucleic acid that has been treated with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid, as required by Claim 1 and its dependent claims 7-**8**, 11-12 and 14-18.

Because the combination of references cited in the instant 103 rejection does not teach the claimed invention, it would not be obvious to combine them. Therefore, reconsideration and withdrawal of the rejection is respectfully requested.

The Office Action states that Claims 1, 7-8, 11-12 and 14-16 are rejected under 35 U.S.C. 103(a) as being unpatentable over Yuan (6,376,210), in view of Prober et al. (Science (1987) 238:336-341).

Applicant traverses the rejection on the grounds that the cited references, either singularly or when combined, do not teach the claimed invention. The claims require that the sample subjected to an analytical procedure is "said treated sample", (see lines 5-6 of Claim 1), that "said sample comprises template nucleic acid and synthetic nucleic acid", (see lines 2-3 of claim 1), and that said sample is "treated with a substance that cleaves template nucleic acid without substantially cleaving said synthetic nucleic acid", (see lines 4-5 of claim 1).

The Office Action states that Yuan et al. teach a method for preparing a nucleic acid sample for an analytical procedure (mutagenesis), said method comprising treating said sample with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid. The Office Action states that Prober et al teaches sequencing using fluorescent chain terminating dideoxynucleotides to produce DNA fragments which are then resolved using polyacrylamide gel electrophoresis.

The Office Action states that it would have been obvious to one of skill at the time the invention was made to have confirmed the identification of the prepared nucleic acid by sequencing by using fluorescent chain terminating dideoxynucleotides coupled with polyacrylamide gel electrophoresis in order to have achieved the benefit of providing a more efficient and effective means of **confirming the identification of the transformed colonies.**

As discussed in the traversal of the preceding 103 rejection, the sample of nucleic acid subjected to the analytical procedures comprising polyacrylamide gel electrophoresis of a sequencing reaction in the stated obviousness rejection, is not the recited "treated sample" required by the instant claims. Instead, in the obviousness rejection, the sample of nucleic acid subjected to the analytical procedure comprising polyacrylamide gel electrophoresis of a sequencing reaction, is nucleic acid that has been isolated from transformed cells. Nucleic acid from transformed cells is distinct from the claimed nucleic acid sample comprising a template and a synthetic nucleic acid that has been treated with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid, as required by Claim 1 and its dependent claims 7-8, 11-12 and 14-16.

Because the combination of references cited in the instant 103 rejection does not teach the claimed invention, it would not be obvious to combine them. Therefore, reconsideration and withdrawal of the rejection is respectfully requested.

The Office Action states that Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Howley et al. (USPN 6,432,926) as applied to claims 1, 7, 11-12 and 14-18 above, and further in view of Sorge (USPN 6,6060,245).

Applicant traverses the rejection on the grounds that the cited references, either alone or when combined, do not teach the claimed invention.

Applicant notes that in response to the 102 rejection of claims 1, 7, 11-12 and 14-18 anticipated by Howley, the instant claims have been newly amended to remove the analytical procedures of gel electrophoresis and Southern Analysis. Since Claim 1 no longer recites a method comprising the analytical procedures taught by Howley et al, and since the teachings of Sorge do not make up the deficiencies of Howley et al., the three cited references can not be combined with the purpose of coming up with the instantly claimed invention.

In view of the claim amendments of claim 1 and for the reasons just discussed above, the combination of cited references can not be used to arrive at the invention of the instant claims. Therefore, reconsideration and withdrawal of the rejection is respectfully considered.

The Office Action states that Claim 13 is rejected under 35 U.S.C. 103(a) as being unpatentable over Dayn et al. (6,245,565) as applied to Claims 1, 7-9 and 11-12, and further in view of Sorge (6,060,245).

Applicant traverses the rejection on the grounds that the cited references, either alone or when combined, do not teach the claimed invention.

The Office Action states that "Dayn et al. teaches analyzing a desired synthetic nucleic acid after cleavage of the template nucleic acid using Dpn I," but that "Dayn et al does not teach cleaving nucleic acid comprising un-modified residues, without substantially cleaving modified residues", and that Dayn et al teaches as above in the 102 rejection of claims 1, 7 and 11-12, a method of preparing a nucleic acid sample for an analytical procedure, said sample comprising

template nucleic acid and synthetic nucleic acid, wherein said template and synthetic nucleic acid comprise DNA, said method comprising treating said sample with a substance that cleaves said template nucleic acid without substantially cleaving said synthetic nucleic acid, and subjecting said treated sample to an analytical procedure, wherein said analytical procedure is selected from the group consisting of gel electrophoresis and Southern blotting.

Applicant notes that the method of the instant claims has been newly amended to remove the recitation of the analytical procedures of "gel electrophoresis and Southern Analysis". Since Claim 1 no longer recites a method comprising the analytical procedures taught by Dayn et al., and since the teachings of Sorge do not make up the deficiencies of Dayn et al., the cited references can not be combined with the purpose of coming up with the instantly claimed invention.

In view of the claim amendments of claim 1 and for the reasons discussed above, the combination of cited references can not be used to come up with the invention of the instant claims. Therefore, reconsideration and withdrawal of the rejection is respectfully considered.

The Office Action states that Claims 20, 22 and 25-26 are rejected under 35 U.S.C. 103(a) as being unpatentable over Chung (USPN 5,683,988), as applied to claims 6, 9, 19 and 23-24 above, and further in view of Bauer et al. (USPN 5,789,166).

Applicant notes that Grocke is mentioned in the text of the rejection but not in the rejection statement. Applicant assumes that Grocke was intended to be referenced as art cited in the instant rejection. Clarification is respectfully requested.

Applicant traverses the rejection on the grounds that the cited references, either alone or when combined, do not teach the claimed invention.

Applicant contends that the method of Chung does not provide for an analysis of the RNA sample as required by the instant claims. Line 3 of independent claim 6, from which the instantly rejected claims depend, recites "immediately prior to the analysis of the RNA sample". In contrast, Chung et al. does not teach that that the RNA was itself analyzed, but instead teaches that the RNA is used as a probe in the analysis of mouse wound tissue:

> "After the transcription reaction is completed, the template DNA was cleaved with DNAse I and precipitated with ethanol to recover the RNA probe as a residual product. The recovered RNA probe was dissolved in 50 .mu.l of 10 mM DTT, 10 mM Tris-HCl, 1 mM EDTA (pH7.6) and then used in hybridization."

and

> "0.5 .mu.g of the probe as obtained above was mixed with 100 .mu.l of a hybridization reaction solution (consisting of 50% deionized formamide, 10 mM Tris-HCl, pH7.6, 200 .mu.g/ml of RNase-free tRNA, 1.times.Denhardt solution, 10% dextran sulphate, 600 mM NaCl, 0.25% SDS) and then 50 to 100 .mu.l of the mixture was loaded on each slide glass to which mouse wound tissue as prepared above 1) was attached, and was subjected to hybridization for 16 to 22 hours while covering the slide glass with cover glass. After the hybridization is completed, the cover glass was removed from the slide glass in 5.times. SSC at 50.degree. C., and the hybridized product was treated with RNase to remove unnecessary signals."

Therefore, Chung does not teach a method that comprises the analysis of the RNA sample, as required by the instantly rejected claims 20, 22 and 25-26.

Nor does Chung teach a method that comprises the cleavage of template nucleic acid with a restriction enzyme, as required by the instantly rejected claims 20, 22 and 25-26.

The Office Action states that:

> " Chung teaches an in vitro transcription reaction carried out using template DNA from transformed E. coli cells (e.g. HB101), wherein following the transcription reaction, the template DNA was cleaved by a ***restriction enzyme*** so that ***only the RNA would be analyzed*** ", (see column 8, lines 12-24), emphasis added.

Applicant respectfully disagrees with this description of Chung's teachings as stated in the Office Action. Specifically, as stated above, Applicant can not find where Chung teaches analyzing RNA. Further, Applicant contends that Chung does not teach that the template DNA was cleaved by a restriction enzyme, but rather that the DNA was cleaved with a nuclease, DNAse I, as discussed above. Applicant notes that Chung teaches in column 8, lines 22-24, that "After the transcription reaction is completed, the template DNA was cleaved with ***DNAse I*** and precipitated with ethanol to recover the RNA probe as a residual product". As discussed above,

DNAse is not a restriction enzyme. Applicant submits that the teachings of Bauer and Gocke can not make up both these differences.

The Office Action characterizes Grocke et al. as:

> "teaching that following a transcription based reaction; the undesired DNA is removed by cleaving DNA with a restriction enzyme, which results in enriching the desired product (e.g.) RNA in an in vitro restriction transcription reaction)(see Figure 2 and column 14). Accordingly, Gocke teaches that it is advantageous to degrade undesired DNA using restriction enzymes".

However, Applicant finds no mention of degrading or removing undesired DNA from an in vitro transcription reaction taught by Gocke. More importantly, Gocke does not teach the ***analysis of the RNA sample*** as required by the instant claims, nor does Gocke teach the use of a restriction enzyme ***after*** the transcription step also required by the instant claims.

In contrast, column 14 of Gocke teaches a method of enriching mutant DNA relative to non mutant DNA from an isolated DNA sample through the ***use of a restriction enzyme before the transcription based amplification step***, according to the method of Kahn as follows:

> "Oncogenes such as p53, p16, BRCA1 and ras exhibit a number of alterations in their DNA sequence that can be identified on the basis of altered restriction enzyme recognition and cleavage. The second step of the invention uses cleavage of normal, non-mutated oncogene DNA by a restriction endonuclease chosen to span one or more of the nucleotides known to be mutated with some frequency in cancers and their precursors. DNA can then be amplified by any of several methods including but not limited to the polymerase chain reaction, the ligase chain reaction, self-sustaining sequence replication and others. Since wild-type DNA has been selectively cleaved by restriction endonuclease digestion, and cleavage prevents DNA amplification, mutant oncogene DNA is relatively enriched following the amplification stage. This ***cycle of cleavage and amplification*** may be repeated to further enrich the test sample for mutant DNA."

The Office Action also states that:

> "Accordingly, in view of the teachings of Gocke and Bauer, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Chung so as to have used DpnI to cleave undesired DNA, ***in order to enrich the analysis of the desired RNA***.", emphasis added.

Applicant is not clear what is meant by the phrase " ***enrich the analysis of the desired RNA"***. However, Applicant is interpreting the phrase as indicating an enrichment of desired RNA, and that the motivation for combining the two references of Chung and Gocke is in order to enrich for the desired RNA. . Clarification is respectfully requested.

Gocke's method enriches for one of at least two types of DNA in a ***DNA*** sample as opposed to enriching for ***RNA.*** However, the Office Action states that the motivation for combining the Grocke reference with the Chung reference is to enrich for the desired RNA. The Applicant contends that Grocke does not teach the enrichment of RNA and therefore cannot be used as a motivation for combining the two references to arrive at the claimed invention. Also Gocke's method of using a restriction enzyme ***before*** a transcription based amplification step, prohibits Grocke from being used in combination with Chung to arrive at the claimed invention. ***Similar to Chung et al., Gocke does not teach the analysis of the RNA sample as required by the instant claims, and therefore Gocke cannot make up this deficiency in the method of Chung.***

Applicant contends that Figure 2 does not contribute to the claimed invention because there is no mention or indication of an in vitro transcription assay in Figure 2 or its legend, and there is no mention or indication of a nucleic acid sample comprising template nucleic acid and synthetic RNA, nor is there any mention or indication that such a nucleic acid sample is treated with a restriction enzyme that cleaves the template nucleic acid and does not substantially cleave the RNA, as required by the instant claims. In contrast Figure 2 depicts only DNA, not RNA, and indicates that restriction enzyme digestion is a way to distinguish between mutated and unmutated DNA, see Figure 2 and see column 14, lines 45-60, which state that "Since wild type DNA has been selectively cleaved by restriction endonuclease digestion , and ***cleavage prevents DNA amplification***, mutant oncogene DNA is relatively enriched following the amplification stage".

The Office Action contends that in view of the teachings by Gocke and Bauer, it would have been obvious to one of skill in the art at the time the invention was made to have used DpnI to cleave undesired DNA in order to enrich the analysis of the desired RNA. However,

Applicant contends that since Grocke does not teach the analysis of the RNA sample as required by the instant claims, and therefore cannot make up this deficiency in the method of Chung. Applicant also contends that since Gocke's teaching of a restriction enzyme before a transcription based amplification step does not teach the enrichment of RNA after the transcription reaction as required by the instant claims, it can not support the motivation asserted by the Office Action for combining the cited references.

Applicants request reconsideration and withdrawal of the rejection on the grounds that the combined references do not arrive at the claimed invention, and because Grocke does not support the motivation asserted by the Office action for combining the cited references.

The Office Action states that claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Gong (J. Biochem. Mol. Biol. (1995) 28 (6):538-545) as applied to claims 6, 9, 19 and 23-24 above, and further in view of Sorge et al. (USPN 6,060,245).

The Office Action states that Gong teaches performing an in vitro transcription reaction, and then cleaving the template DNA, but not substantially cleaving the synthetic RNA, thereby enriching the RNA for further analysis.

Applicant traverses the rejection on the grounds that the combined references do not arrive at the claimed invention, with all its recited limitations.

The Office Action states that " Gong does not teach cleaving template DNA comprising un-modified residues, ***without substantially cleaving modified residues***". The Office Action further states that "Sorge teaches generating or analyzing desired nucleic acid sequences by cleaving nucleic acids comprising modified residues, ***without substantially cleaving un-modified residues...***".

Applicant notes that according to the Office Action, neither of the references cited in the instant rejection (Chung or Sorge) teach the recited limitation of claim 21 which is "wherein said restriction enzyme specifically cleaves nucleic acid comprising un-modified residues, ***without substantially cleaving modified residues***". Because neither reference teaches this

recited limitation of claim 21, Applicant contends that considering these two references, either independently or when combined, does not produce the invention of claim 21.

The Office Action states that" in view of the teachings of Sorge, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Chung so as to have cleaved the undesired nucleic acid ***without substantially cleaving unmodified residues***", (emphasis added). However, as noted above, combining the references of Gong and Sorge as described in the office action, does not meet all the recited limitations of claim 21, including the limitation of "wherein said restriction enzyme specifically cleaves nucleic acid comprising un-modified residues, ***without substantially cleaving modified residues***". Accordingly Applicant respectfully requests reconsideration and withdrawal of the instant rejection.

The Office Action states that claim 21 is rejected under 35 U.S.C. 103(a) as being unpatentable over Chung (USPN 5,683,998) as applied to claims 6, 9, 19 and 23-24 above, and further in view of Sorge et al (USPN 6,060245).

Applicant traverses the rejection on the grounds that the combined references do not arrive at the claimed invention, with all its recited limitations.

The Office Action states that Chung teaches performing an in vitro transcription reaction, and then cleaving the template DNA, but not substantially cleaving the synthetic RNA, thereby enriching the RNA for further analysis.

The Office Action states that "Chung does not teach cleaving template DNA comprising un-modified residues, ***without substantially cleaving modified residues***". The Office Action further states that "Sorge teaches generating or analyzing desired nucleic acid sequences by cleaving nucleic acids comprising modified residues, ***without substantially cleaving un-modified residues...***".

Applicant notes that according to the Office Action, neither of the references cited in the instant rejection (Chung or Sorge) teach the recited limitation of claim 21 which is "wherein

said restriction enzyme specifically cleaves nucleic acid comprising un-modified residues, ***without substantially cleaving <u>modified</u> residues***". Because neither reference teaches this recited limitation of claim 21, Applicant contends that considering these two references, either independently or when combined, does not produce the invention of claim 21.

The Office Action states that" in view of the teachings of Sorge, it would have been obvious to one of ordinary skill in the art at the time the invention was made to have modified the method of Chung so as to have cleaved the undesired nucleic acid ***without substantially cleaving <u>unmodified</u> residues***", (emphasis added). However, as noted above, combining the references of Chung and Sorge as described in the office action, does not meet all the recited limitations of claim 21, including the limitation of "wherein said restriction enzyme specifically cleaves nucleic acid comprising un-modified residues, ***without substantially cleaving <u>modified</u> residues***". Accordingly Applicant respectfully requests reconsideration and withdrawal of the instant rejection.

Conclusion

Applicants submit that in view of the foregoing remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicants respectfully request the withdrawal of rejections over the claims of the present invention.

Respectfully submitted,

Date: 5/26/04

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Tel: 617-239-0100

Product Contents

RQ1 RNase-Free DNase:

Part No.	Size (units)
M610A	1,000

Part# 9PIM610

Revised 2/04

Description: RQ1 (RNA-Qualified) RNase-Free DNase is a DNase I (endonuclease) that degrades both double-stranded and single-stranded DNA, producing 3´-OH oligonucleotides (1). (RQ1 RNase-Free DNase may be used in applications where maintaining the integrity of the RNA is critical.) This DNase is suited for applications such as nick translation (2), production of random fragments (3), cleavage of genomic DNA for footprinting (3), removal of DNA template after in vitro transcription (4), and removal of DNA from RNA samples prior to applications such as RT-PCR (5).

In the presence of Mg^{2+}, DNase I attacks each strand of DNA independently, and the sites of cleavage are distributed in a statistically random fashion (6). In the presence of Mn^{2+}, DNase I cleaves both strands of DNA at approximately the same site to yield fragments with blunt ends or protruding termini of one or two nucleotides in length (6).

10X Reaction Buffer (M198A): The RQ1 DNase 10X Reaction Buffer provided with this enzyme has a composition of 400mM Tris-HCl (pH 8.0), 100mM $MgSO_4$ and 10mM $CaCl_2$.

Enzyme Storage Buffer: RQ1 DNase is supplied in 10mM HEPES (pH 7.5), 50% glycerol (v/v), 10mM $CaCl_2$ and 10mM $MgCl_2$.

Heat Inactivation: 10 minutes at 65°C in the presence of Stop Solution.

Inhibitors: EGTA; EDTA (7); salt concentrations >100mM will reduce DNase activity.

Molecular Weight: 31,000 Daltons.

Requirement: Ca^{2+} and Mg^{2+} or Mn^{2+} (7).

Source: Bovine pancreas.

Storage Temperature: Store at –20°C. Avoid exposure to frequent temperature changes. See the expiration date on the Product Information Label.

Stop Solution (M199A): 20mM EGTA (pH 8.0).

Unit Definition: One unit of RQ1 RNase-Free DNase is defined as the amount required to completely degrade 1µg of lambda DNA in 10 minutes at 37°C in 50µl of a buffer containing 40mM Tris-HCl (pH 7.9), 10mM NaCl, 6mM $MgCl_2$ and 10mM $CaCl_2$. Under these assay conditions one unit of RQ1 DNase activity is approximately equal to one Kunitz unit. See the unit concentration on the Product Information Label.

Usage Notes:

1. This DNase solution does not contain an RNase inhibitor. Observe caution in handling the product to ensure against contaminating it with RNase.
2. Under different buffer conditions the amount of DNase required to completely digest a given amount of DNA may need to be empirically determined. For example, salt concentrations >100mM will reduce DNase activity.

Quality Control Assays

Contaminant Activity

RNase Assay: To test for RNase activity, 50ng of [^{3}H]RNA is incubated with 5 units of RQ1 RNase-Free DNase in Transcription Optimized 1X Buffer (Cat.# P1181, diluted fivefold) for 1 hour at 37°C, and the release of radiolabeled nucleotides is monitored by scintillation counting of TCA-soluble material. The minimum passing specification is <3% release.

Promega Corporation

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Part# 9PIM610
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Promega

Usage Information

I. DNase Treatment of RNA Samples Prior to RT-PCR

1. Set up the DNase digestion reaction as follows:

RNA in water or TE buffer	1–8µl
RQ1 RNase-Free DNase 10X Reaction Buffer	1µl
RQ1 RNase-Free DNase (see Note 1)	1u/µg RNA
Nuclease-free water to a final volume of	10µl

2. Incubate at 37°C for 30 minutes.

   Note: If analyzing RNA samples by gel electrophoresis, perform a phenol:chloroform extraction and ethanol precipitation before loading the samples on the gel, because salts in the RQ1 DNase Reaction Buffer and Stop Solution cause aberrant migration of RNA on gels. Steps 3 and 4 may be omitted if a phenol:chloroform extraction is performed.

3. Add 1µl of RQ1 DNase Stop Solution to terminate the reaction.
4. Incubate at 65°C for 10 minutes to inactivate the DNase.
5. Add all, or a portion of, the treated RNA to the RT-PCR reaction. See the *Access RT-PCR System*[a] *Technical Bulletin* #TB220.

Notes:

1. Use 1 unit of RQ1 RNase-Free DNase per microgram of RNA. For smaller amounts of RNA, use 1 unit of RQ1 RNase-Free DNase per reaction.
2. The RQ1 RNase-Free DNase digestion contains a final concentration of 10mM $MgSO_4$. When adding the DNase-treated RNA to an RT-PCR reaction, carryover of magnesium must be considered. For example, the addition of 1µl of treated RNA to a 50µl RT-PCR reaction will raise the magnesium concentration by 0.2mM, and the addition of 5µl of treated RNA will raise the magnesium concentration by 1mM. The requirement for magnesium may be different in the RQ1 DNase digestion step and in the amplification reaction.
   - RQ1 DNase activity increases as the Mg^{2+} concentration increases up to 5–10mM. At a concentration of 1mM Mg^{2+}, RQ1 DNase is expected to be at least fourfold less active than at the optimal Mg^{2+} concentration.
   - For some templates, the yield from the amplification reaction is highly dependent on the Mg^{2+} concentration, and the optimal Mg^{2+} concentration may be as low as 1mM.

   If an increased Mg^{2+} concentration is not tolerable in the amplification reaction, the following alternatives may be used.
   - The RQ1 RNase-Free DNase 10X Reaction Buffer may be diluted 1:10 with 400mM Tris (pH 8.0), 10mM $CaCl_2$ prior to DNase digestion. (Note that, under these conditions, the RQ1 DNase will be approximately fourfold less active than under standard reaction conditions.)
   - An alternative DNase reaction buffer may be used (such as the RT or PCR reaction buffer) if that buffer contains at least 1mM Mg^{2+}.
   - The RNA sample may be diluted in water prior to RT-PCR allowing dilution of the $MgSO_4$ to a concentration that is compatible with this application.
   - The RNA may be purified with a standard phenol:chloroform extraction followed by an ethanol precipitation.

II. Other Applications

RQ1 RNase-Free DNase may be used in a number of other applications where maintaining the integrity of RNA is important. These include in vitro transcription, nick translation and DNase I footprinting.

A. In Vitro Transcription

To remove template DNA, RQ1 RNase-Free DNase may be added directly to the transcription reaction. Please refer to the *Riboprobe® in vitro Transcription Systems*[b,c] *Technical Manual* #TM016 (4) for specific protocol information.

B. Nick Translation

For protocol information on the use of RQ1 RNase-Free DNase for this application, please refer to the *Protocols and Application Guide* (2).

C. Transcription Factor DNase I Footprinting

RQ1 RNase-Free DNase is a component of the Core Footprinting System and may be used in footprinting experiments to determine whether a gene of interest contains a specific DNA binding protein binding site. For specific protocol information, please refer to the *Core Footprinting System Technical Bulletin* #TB137 or see reference 8.

D. Production of Random Fragments

For protocol information on the use of DNase I for random fragmentation of DNA, see reference 6.

Note: Under different buffer conditions, the amount of RQ1 RNase-Free DNase required to completely digest a given amount of DNA must be empirically determined. For example, salt concentrations >100mM will result in reduction of DNase activity. Ca^{2+} and Mg^{2+} are essential for RQ1 DNase activity.

III. References

1. Moore, S. (1981) Pancreatic DNase In: *The Enzymes*, Volume 14A, P.D. Boyer, Ed., Academic Press, New York, 281.
2. *Protocols and Applications Guide* (1996) Promega Corporation.
3. Cobianchi, F. and Wilson S.H. (1987) *Meth. Enzymol.* **152**, 94–110.
4. *Riboprobe® in vitro Transcription Systems Technical Manual* #TM016, Promega Corporation.
5. *Access RT-PCR System and Access RT-PCR Introductory System Technical Bulletin* #TB220, Promega Corporation.
6. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) *Molecular Cloning: A Laboratory Manual* 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor, New York.
7. Ausubel, F.M. (1994) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 3.12.
8. Ausubel, F.M. (1994) *Current Protocols in Molecular Biology*, John Wiley and Sons, New York, 12.4.

[a]The PCR process is covered by patents issued and applicable in certain countries. Promega does not encourage or support the unauthorized or unlicensed use of the PCR process. Use of this product is recommended for persons that either have a license to perform PCR or are not required to obtain a license.

[b]U.S. Pat. No. 5,552,302. European Pat. No. 0 422 217. Australian Pat. No. 646803 and Japanese Pat. Nos. 3009458 and 3366596 have been issued to Promega Corporation for the methods and compositions for production of human recombinant placental ribonuclease inhibitor.

[c]U.S. Pat. Nos. 4,966,964, 5,019,556 and 5,266,687. Australian Pat. Nos. 616881 and 641261 and other pending and issued patents, which claim vectors encoding a portion of human placental ribonuclease inhibitor, are exclusively licensed to Promega Corporation.

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